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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Richard Wurtman, Ph.D. Examiner: L. E. Crane, Ph.D.

Serial No.: 09/363,748

Art Unit: 1623

Filed: July 30, 1999

For: METHODS FOR TREATING CYTIDINE LEVELS AND METHODS FOR TREATING CYTIDINE-DEPENDENT HUMAN DISEASES

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
Washington, DC 20231
Sir:

I, Prof. Richard J. Wurtman, Ph.D., hereby make the following declaration:

1. I am a co-inventor of the above-identified application and currently hold the positions Cecil H. Green Distinguished Professor MIT, and Director, General Clinical Research Center, Harvard-MIT Division of Health Science and Technology.

2. I understand that this application is being amended to claim to a method for enhancing memory, by administering to a subject in need thereof an effective amount of a uridine phosphate. I understand that at the interview held on March 28, 2003, Examiner Crane took the position that the phrase "a uridine phosphate" could indicate phosphorylation on any of three different ribose ring positions.

3. In my opinion, a person of skill in the art would interpret the phrase "a uridine phosphate" and the phrase "uridine phosphates" (page 13, line 18 of the present specification) as referring to the three naturally occuring 5-prime phosphates of uridine. Uridine is phosphorylated by ubiquitous pyrimidine nucleoside kinase

enzymes ("uridine kinase") to form uridine monophosphate (UMP). These enzymes attach the phosphate moiety to the 5-prime hydroxyl on the ribose ring of the uridine molecule. UMP can be further phosphorylated to form uridine 5-prime di-phosphate (UDP) by the enzyme(s) pyrimidine nucleoside monophosphate kinase. UDP can be converted to uridine 5-prime tri-phosphate (UTP) by the nucleoside diphosphokinase enzyme(s).

In each case, an additional phosphate is added to one of the hydroxyls on the UMP or UDP phosphate moiety and the final di- or tri-phosphorylated products are still 5-prime compounds. All three enzymes can also catalyze the phosphorylation of cytidine/CMP/CDP, however, uridine is the preferred substrate for all of them.

Supporting documentation is attached: Merck Index pp. 1684 – 1685; and Volume LI of METHODS in ENZYMOLOGY, "Purine and Pyrimidine Nucleotide Metabolism," pp. 305 – 306, 318, 327, 329 - 330, 371 and 375, Hoffee & Jones ed. (1978).

4. Effect of Uridine Monophosphate (UMP.2Na) on Memory in Memory-Impaired Rats

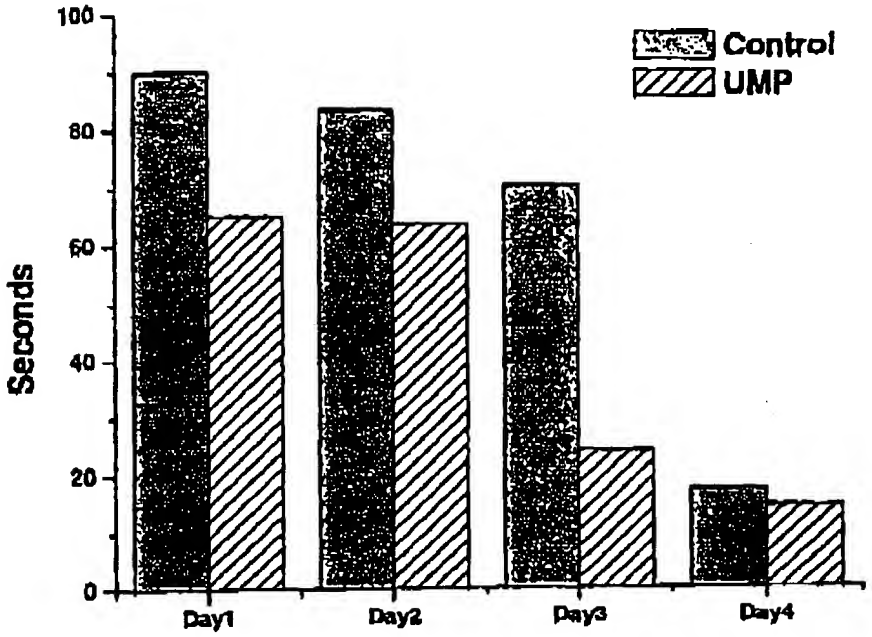
Prior studies performed at MIT and elsewhere had shown that both aging and social deprivation can generate cognitive/memory impairments in rats. In the present study, conducted under my supervision and control, six 12-month-old rats were housed individually and deprived of most social stimulation for six weeks. During this period the animals had access either to a) a control diet containing some choline (1 g/kg) but no uridine or compounds that break down to form uridine; or b) the experimental diet, i.e., the control diet to which sufficient UMP was added so that the animals would consume 500 mg/kg/day. This was done by weighing them every 3 days, and, as the animals grew, increasing the amount of UMP available in the diet.

5. After the six week isolation period, memory/cognition was tested for four days, by placing the animals in a Morris Water Maze and determining how quickly they learned to find the platform. In this test, a platform is located below the surface of the water which is not visible to the swimming animals. Once they locate and stand on the platform they can stop swimming.

6. On each of the four test days, each animal was tested 4 times. The

graph below shows the average number of seconds it took the animals in each group to find the platform. The animals consuming the control diet learned more slowly, as shown by the fact that their performance on the 3rd test day wasn't much different from that on the first day. In contrast, the animals on the UMP-containing diet learned more quickly on all days, the difference being greatest on the third test day. By the 4th test day the control animals also had learned this task. In my opinion this data indicates that UMP displayed a memory-enhancing effect in these rats.

Effect of Oral UMP·2Na$^+$ on Memory Retention



12 month old rats (N=3 per group) weighing 500 g consumed a control diet or this diet fortified with UMP·2Na$^+$ (providing 500 mg/kg/day) for 6 weeks. They were then tested, using a Morris Water Maze, for 4 days.

7. Percent Increase in Gerbil Brain Content of Kennedy Pathway Intermediates after Oral UMP

The following experiments, conducted under my supervision and control, provide biochemical data showing that when gerbils receive UMP their brains synthesize more of the rate-limiting intermediate needed to form membrane phosphatidylcholine, i.e., CDP-choline. The animals received a UMP dose equivalent to 250 mg/kg citicoline, which is known to increase brain phosphatidylcholine synthesis and levels.

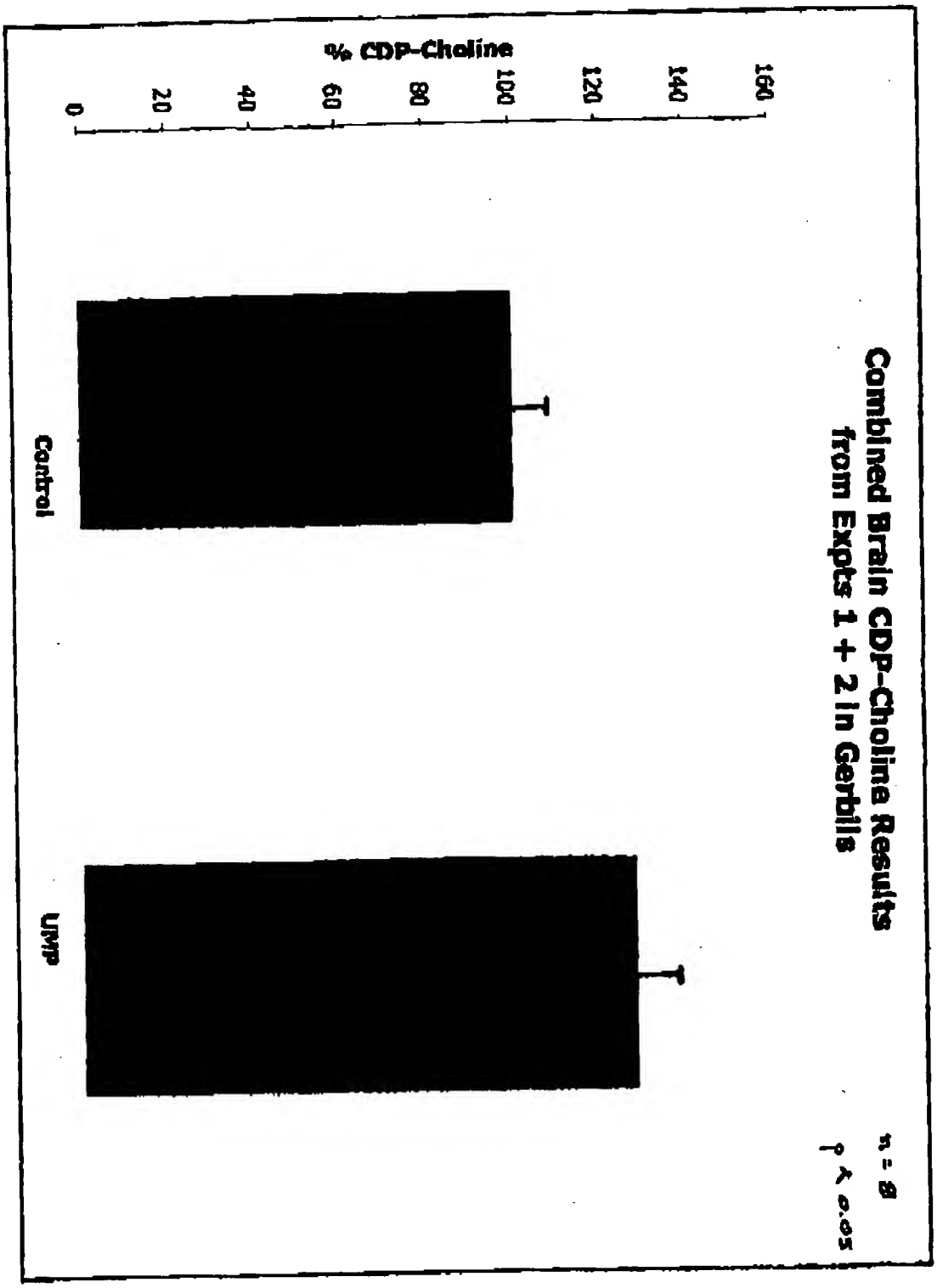
8. Gerbils with free access to food and water were given disodium UMP (1 mmol/kg) orally, by gavage. Control animals did not receive any UMP supplement. Sacrifice by decapitation under Telazol anesthesia (60 – 80 mg/kg) occurred at either 1 or 3 hours post-treatment. Two separate experiments were performed and the data pooled so that sample size was n=8.

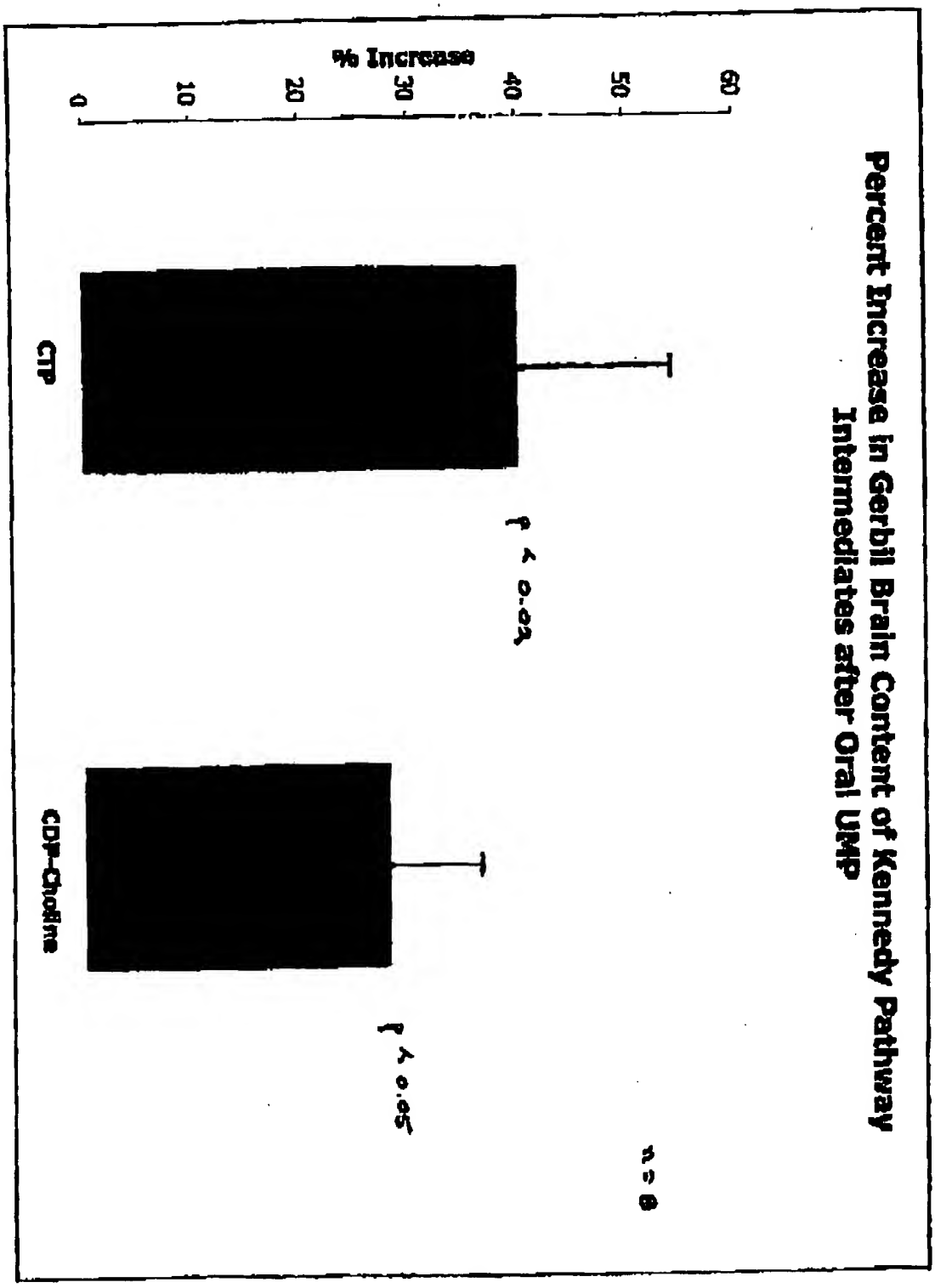
9. Brains were rapidly removed and frozen on dry ice before homogenization in cold 80% methanol. After centrifugation, the supernatants were extracted with chloroform. A portion of the aqueous layer was lyophilized and redissolved in 400 ul of water for HPLC analysis.

10. Samples were analyzed on a Beckman System Gold equipped with an Alltech ion exchanger column (APS-2, 3 micron packing, 4.5 x 150 mm), column heater at 37 °C, and UV detector at 280 nm.

11. For CTP analysis, compounds were eluted using an isocratic buffer (75 mM sodium phosphate, pH 3.6) flowing at 1 ml/min. For CDP-choline analysis, compounds were eluted using a gradient system starting with 2 mM phosphoric acid (pH 2.84) flowing at 1 ml/min; after 5 minutes began a convex gradient to 500 mM sodium phosphate (pH 4.3) over a 20-minute period. The column was then re-equilibrated with the initial buffer for 20 minutes before injecting the next sample.

12. In the first experiment, the absolute values for CDP-choline level were 6.8, 6.2 and 5.8 nanomoles/gram (control group); and 9.4, 9.2, 7.2 and 5.9 nanomoles/gram (test group). The second experiment showed similar basal levels and trends in brain chemistry. Pooled data from the two experiments (n=8) were statistically significant and showed that after the animals received UMP, their brain CTP and CDP-choline levels were much higher ($p<0.02$ and $p<0.05$, respectively).


% CDP-Choline
0
20
40
60
80
100
120
140
160
Control
UMP
Combined Brain CDP-Choline Results
from Expts 1 + 2 in Gerbils
n = 8
p < 0.05


Percent Increase in Gerbil Brain Content of Kennedy Pathway Intermediates after Oral UMP
% Increase
0
10
20
30
40
50
60
CTP
CDP-Choline

13. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Oct. 22, 2003

By: _____
Prof. Richard J. Wurtman